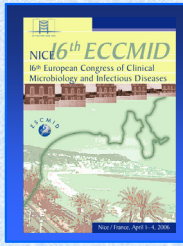


Meropenem and cloxacillin are active against MRSA clinical isolates (including VISA) in acidic broth and in THP-1 macrophages.

S. Lemaire,[1] F. Van Bambeke,[1] P.M. Tulkens,[1] M.P. Mingeot-Leclercq,[1] J. Coyette,[2] Y. Glupczynski[3]

[1]Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Brussels;
[2]Centre d'ingénierie des protéines, Université de Liège, Liège; [3]Laboratoire de microbiologie, Cliniques universitaires UCL Mont-Godinne, Yvoir; Belgium

Mailing address:
P.M. Tulkens
Pharmacologie cellulaire et moléculaire
UCL 73.70 av. Mounier 73
1200 Brussels - Belgium
tulkens@facm.ucl.ac.be

P 1534

Abstract

Objectives: Exposure of Methicillin-resistant *S. aureus* (MRSA) to acid pH restores its susceptibility to beta-lactams (Sabath and *al.*, AAC, 1972). In macrophages, *S. aureus* is mainly confined within phagolysosomes where the pH is acidic. We showed that meropenen (MEM) displays similar intracellular activity against MRSA ATCC 33591 and MSSA ATCC 25923 in macrophages. In the present study, we have investigated the intraphagocytic activity of MEM and cloxacillin (CLX) against 3 MRSA clinical isolates (including one VISA strain), in comparison with the reference MRSA (ATCC 33591) and MSSA (ATCC 25923) strains.

Methods: MIC's were determined in MHB (plus NaCl 2%) by micro-dilution method. mecA expression was examined at neutral and acidic pH by a semi-quantitative RT-PCR (16 S rRNA as housekeeping gene). Intracellular activity was assessed in human THP-1 macrophages exposed to extracellular concentrations equivalent to human Cmax (total drug; MEM: 50 mg/L; CLX: 8 mg/L) by examining the decrease in cell-associated CFU after 24 h from the original, post-phagocytosis inoculum (controls [no antibiotic]; approx. 1 log CFU increase).

Results: The table shows the MICs (in broth) at neutral and acid pH and the intracellular activity for the 5 strains studied.

Strains		MEM			CLX		
		MIC (mg/L)		Δ CFU	MIC (mg/L)		Δ CFU
		pH 7.4	pH 5.5		pH 7.4	pH 5.5	
MSSA	ATCC 25923	0.125	0.125	-0.9±0.1	0.125	0.03	-0.7±0.1
MRSA	ATCC 33591	16	0.125	-0.6±0.1	128	0.06	-0.5±0.1
	N4120032	2	0.06	-0.8±0.1	1	0.06	-0.6±0.1
	N4120210	2	0.06	-0.6±0.1	1	0.06	-0.4±0.0
	VISA, NRS18	8	0.06	-0.6±0.1	8	0.03	-0.5±0.1

In ATCC 33591, mecA expression was similar for bacteria maintained in broth at pH 7.4 or 5.5 (O.D. mecA/O.D. 16 S rRNA : 0.20 ± 0.1 vs. 0.24 ± 0.1).

Conclusions : The intracellular environment markedly enhances the activity of beta-lactams against MRSA, probably through exposure to acid pH, although the latter does not affect mecA expression.

Background

MRSA, a major source of nosocomial and community-acquired infections, show high level of resistance to β-lactams, in relation with the expression of a modified PBP (PBP2a) taking over the biosynthetic function of conventionnal PBPs and displaying low affinity for β-lactam drugs.

β-lactams activity against MRSA is however restored at acidic pH[1]. This may be of interest for infections by *S. aureus* developing in acidic compartments. In particular, *S. aureus* has the potential of surviving within the phagolysosomial compartments of phagocytic cells (where pH is acidic). In macrophage cells, we recently showed that β-lactams do exert intracellular activity against MSSA[2].

Aim of the study

- To study the influence of acid pH on the activity of MEM and CLX against MSSA and MRSA and on the expression of mecA
- To determine the intracellular activity of MEM and CLX

Methods

• **Susceptibility** was assessed by broth micro-dilution method, in MHB supplemented with NaCl 2 % and adjusted to pH 7.4 or 5.5.

• **Intracellular activity** was studied in THP-1 macrophages[2]. Briefly, cells were infected with pre-opsonised bacteria (1h, 37°C), washed with PBS for the elimination of non-adherent and non-phagocytosed bacteria, and finally resuspended for 24 h in fresh medium supplemented with MEM or CLX (extracellular concentration : 0.01 to 1000 x MIC pH 5.5).

•SQ-RT PCR protocol was followed for the **determination of mecA expression**. Exponential cultures of MRSA (ATCC 33591; O.D. = 0.3) in neutral and acidic broth were harvested and lysed upon the action of lysostaphin (100 mg/L) and lysozyme (3 g/L). Extraction of RNA was then performed using Rneasy Mini Kit (Qiagen, Germany) and RNA integrity was checked after addition of RQ1 Rnase-free Dnase I (Promega Corporation, USA). Reverse transcription was achieved using AMV Reverse Transcriptase system (Promega, USA) and followed by PCR amplification (mecA and 16s RNA as housekeeping gene [3]) in a Gene Thermal Cycler (Biorad, USA) with the following steps : 96°C for 10 min. ; [92° for 60 sec., 50°C for 60 sec., 72°C for 90 sec(n=30)] and 72°C for 10 min. Each RT-PCR product was visualized by Ethidium Bromide staining and densitometric analyses were performed using Image J software (version 1.3.4, available from http://rsbweb.nih.gov/ij/) .

References

1. Sabath et al., AAC, 1972, 2 (5): 350-355.
2. Lemaire et al., JAC, 2005, 55 (6): 897-904.
3. Hanssen et al., AAC, 2004, 48 (1): 285-296.

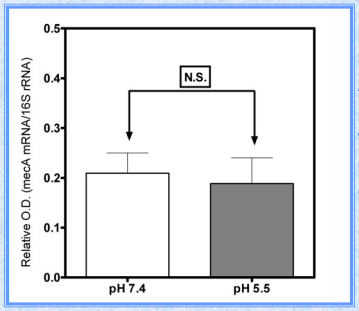
Results

MICs in broth

		MEM		CLX	
		pH 7.4	pH 5.5	pH 7.4	pH 5.5
MSSA	25923	0.125	0.125	0.125	0.03
MRSA	33591	16	0.125	128	0.06
	N4120032	2	0.06	1	0.06
	N4120210	2	0.06	1	0.06
& VISA	NRS18	8	0.06	8	0.03

Acidic medium restores the sensitivity of MRSA to MEM and CLX

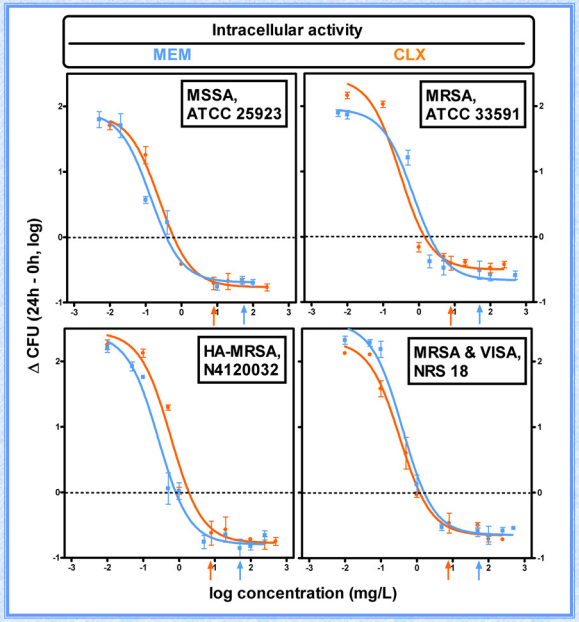
Expression of *mecA* in broth



Acidic pH does not influence the expression of the *mecA* gene.

Fig.1. Expression of *mecA* in MRSA exposed to neutral or acidic broth

Intracellular activity of MEM and CLX :



MEM and CLX display similar dose-dependent activity against intracellular MSSA and all the MRSA tested, including a VISA strain.

Fig.2. Concentration-killing curves of CLX and MEM against MSSA and MRSA in THP-1 macrophages.

The abscissa shows the initial concentration of antibiotics (log_{10}) and the arrow on the x axis points the multiples of MIC corresponding to the human Cmax (8 and 50 mg/L for CLX and MEM, respectively). The ordinate shows the change in CFUs (log_{10}) per mg of cell protein observed after 24 h incubation in comparison with the original inocula (horizontal broken lines). All values are mean ± SEM (n=3).

Conclusions

- Intracellularly, MEM and CLX are as active against MSSA than against all the MRSA tested, probably in relation with their increased activity in acidic milieu
- Increased activity at acidic pH is not related to a change in mecA expression.

A copy of this poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters.htm